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     (FILE 'HOME' ENTERED AT 18:42:01 ON 02 JUN 2004)

     FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 18:42:18 ON 02 JUN
     2004
L1           492 S (DNA OR NUCLEIC(W)ACID OR CDNA OR POLYNUCLEOTIDE)(6A)ANTITRYP
L2        827031 S (ENHANC? OR INCREAS? OR PROMOT?)(5A)(DELIVER? OR ACTIVIT?)
L3            14 S L1 AND L2
L4             9 DUP REM L3 (5 DUPLICATES REMOVED)

=> d bib ab 1-9 l4

L4   ANSWER 1 OF 9  CAPLUS  COPYRIGHT 2004 ACS on STN
AN   2003:133119  CAPLUS
DN   138:175888
TI   Electroporative delivery of polynucleotides and other molecules to organs
IN   Malone, Robert W.; Schmid, Ralph; Kubisa, Bartosz; Uduehi, Aima; Ayuni,
     Erick Lawir; Drabick, Joseph; Glasspool-Malone, Jill
PA   USA
SO   PCT Int. Appl., 52 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.         KIND  DATE           APPLICATION NO.  DATE
     ---------------    ----  --------       ---------------  --------
PI   WO 2003013615      A1    20030220       WO 2002-US24285  20020802
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
             GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
             LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
             PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
             UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU,
             TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
             CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
             PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
             NE, SN, TD, TG
PRAI US 2001-310239P   P    20010807
AB   The present invention relates to the electroporative delivery of a
     polynucleotide or other bioactive mol., such as a drug or therapeutic
     protein, into cells of an organ, without injecting the mol. directly into
     the organ.  Instead, the mol. is delivered by means such as instillation
     or catheterization through an epithelially- or endothelially-lined lumen
     connected to the organ.  Thus, the inventive method combines administering
     a mol. to an organ and electroporation of that organ to provide a method
     which facilitates entry of a mol. into cells of an organ while preserving
     organ function and integrity.  For example, a 500 μL of plasmid solution,
     with or without triammonium salt of aurintricarboxylic acid (ATA), was
     administrated by endotracheal tube to lung of rats.  Electroporation of
     lung tissue with 8 pulses of 300 V/cm field strength, each 20 milli-sec in
     duration, at a frequency of 1 Hz provided for transfection while
     preserving lung function.
RE.CNT  3     THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4   ANSWER 2 OF 9  CAPLUS  COPYRIGHT 2004 ACS on STN
AN   2002:929846  CAPLUS
DN   139:78578
TI   Effects of native and cleaved forms of α1-antitrypsin on ME 1477
     tumor cell functional activity
AU   Zelvyte, Inga; Sjoegren, Hans-Olov; Janciauskiene, Sabina
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CS Wallenberg Laboratory, Department of Medicine, Sweden University Hospital Malmoe, Malmoe, 20502, Swed.
SO Cancer Detection and Prevention (2002), 26(4), 256-265
CODEN: CDPRD4; ISSN: 0361-090X
PB Elsevier Science B.V.
DT Journal
LA English
AB Tumor cells synthesize and release a variety of substances, including proteases and protease inhibitors involved in cell growth and proliferation. α1-Antitrypsin (AAT) is a serine proteinase inhibitor synthesized primarily in the liver, but also in extra-hepatic tissues and cells, including tumor cells. AAT exists not only in a native, active inhibitory form, but also in several, non-inhibitory forms, such as cleaved and/or degraded. This study was designed to investigate the synthesis of AAT by melanoma cells, ME 1477, and the effects of native, cleaved and C-terminal fragment of AAT (C-36) on cell functional activity. We found that ME 1477 cells synthesize and secrete AAT with the same apparent mol. mass as described for AAT purified from plasma, but with no measurable inhibitory activity. As determined by Western blot after immunopptn. of [32S]-labeled AAT, exogenous native or modified forms of AAT added to the cells at a concentration of 10 μM did not change AAT synthesis. Moreover, cells exposed to native AAT show decreased [3H]-thymidine incorporation by 53% and tissue inhibitor of metalloproteinases (TIMP)-1 levels by 36%. In contrast, cells treated with C-36 peptide significantly **increased** metalloproteinase **activity**, and [3H]-thymidine incorporation by 35%. Specifically, pro-collagenase-1 levels were found to be increased by 1.4-fold and decreased by 1.5-fold in cells treated with C-36 peptide and native AAT, resp. Cleaved form of AAT had no significant effects on parameters measured. Data obtained from this study suggest that specific forms of AAT have multiple effects on tumor cell viability and play diverse roles in tumorigenesis.
RE.CNT 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2001:545533 CAPLUS
DN 135:127170
TI Method for nucleic acid transfection of cells
IN Bennett, Michael J.; Rothman, Stephan S.; Nantz, Michael H.
PA Genteric, Inc., USA
SO PCT Int. Appl., 68 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001052903	A1	20010726	WO 2001-US1803	20010119
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 6372722	B1	20020416	US 2000-487089	20000119
	US 2001051610	A1	20011213	US 2001-766320	20010118
	US 6624149	B2	20030923		
	EP 1250156	A1	20021023	EP 2001-908634	20010119
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

JP 2003520253 T2 20030702 JP 2001-552950 20010119
PRAI US 2000-487089 A 20000119
US 2001-766320 A 20010118
WO 2001-US1803 W 20010119
AB The present invention describes methods for introducing nucleic acids into a target cell using a transition metal enhancer. A mixture containing nucleic acid and a transition metal enhancer is exposed to cells. The nucleic acid is taken up into the interior of the cell with the aid of the transition metal enhancer such as cobalt. Since nucleic acids can encode a gene, the method can be used to replace a missing or defective gene in the cell. The method can also be used to deliver exogenous nucleic acids operatively coding for proteins that are secreted or released from target cells, thus resulting in a desired biol. effect outside the cell. Alternatively, the methods of the present invention can be used to deliver exogenous nucleic acids into a target cell that are capable of regulating the expression of a predetd. endogenous gene. This can be accomplished by encoding the predetd. endogenous gene on the nucleic acid or by encoding the nucleic acid with a sequence that is the Watson-Crick complement of the mRNA corresponding to the endogenous gene.
RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1997:247860 CAPLUS
DN 126:229615
TI **Enhanced** artificial viral envelopes for cellular **delivery** of therapeutic substances
IN Conary, Jon T.; Schreier, Hans
PA Advanced Therapies, Inc., USA; Conary, Jon T.; Schreier, Hans
SO PCT Int. Appl., 108 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9704748	A2	19970213	WO 1996-US12750	19960801
WO 9704748	A3	19970529		
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG				
AU 9666914	A1	19970226	AU 1996-66914	19960801
PRAI US 1995-1738P	P	19950801		
US 1995-2580P	P	19950821		
US 1995-690613	A2	19960731		
US 1996-690613	A	19960731		
WO 1996-US12750	W	19960801		

AB This invention provides artificial viral envelopes and other lipid vesicles that encapsulate therapeutic substances, such as expression vectors, targeted to mammalian cells. Polynucleotides may be packed into the envelopes by compressing them beforehand with a short peptide with a predominant pos. charge. The compression step not only facilitates encapsulation, it also increases the number of vesicles containing nucleic acid, minimizes the amount of free nucleic acid, and may also increase the size and complexity of plasmids that can be encapsulated. The vesicles may be provided with a tissue-targeting component that helps direct it towards certain tissue sites in an animal. The vesicles may also be provided with a fusogenic component that facilitates delivery of the therapeutic substance into the cell. The materials and reagents of this invention are effective, for example, in increasing expression of model proteins in both

isolated cells and intact animals, and are expected to be useful for gene therapy.

L4 ANSWER 5 OF 9 MEDLINE on STN DUPLICATE 1
AN 95040398 MEDLINE
DN PubMed ID: 7952657
TI Regulation of the alpha 1-antitrypsin gene and a disease-associated mutation in a related enhancer sequence.
CM Erratum in: Am J Respir Crit Care Med 1995 Mar;151(3 Pt 1):926
AU Kalsheker N A; Morgan K
CS Department of Clinical Chemistry, University Hospital, Queen's Medical Centre, Nottingham, United Kingdom.
SO American journal of respiratory and critical care medicine, (1994 Dec) 150 (6 Pt 2) S183-9. Ref: 30
Journal code: 9421642. ISSN: 1073-449X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199412
ED Entered STN: 19950110
Last Updated on STN: 19980206
Entered Medline: 19941228
AB Ten years ago a search was initiated for **DNA** variation in the alpha-1-**antitrypsin** gene (alpha 1-AT) to determine whether there were mutations more commonly associated with patients who had chronic obstructive airways disease (COAD) than with healthy individuals. Using the conventional approach of Southern blotting and searching for restriction fragment length polymorphisms, we identified a potentially useful polymorphism that resulted in the loss of a recognition site for the restriction enzyme, Taq I. The polymorphism occurred in about 17% of patients with COAD and about 5% of the general population ($p = 0.0016$). The normal sequence in the 3' flanking region of the alpha 1-antitrypsin gene had to be characterized, as it was not known. On the basis of homology, a number of closely clustered sequence motifs demonstrating the characteristics of an enhancer were identified that would potentially increase the transcription and expression of alpha 1-antitrypsin. The normal Taq I sequence occurred in a motif that demonstrated homology to a DNA sequence for octamer transcription factors. The mutation was characterized by in vitro amplification of the region and direct sequencing as a G to A transition (Taq I site TCGA < TCAA). Specific binding of nuclear proteins by gel-shift analysis and DNase I footprinting and **increased** in vivo transcriptional **activity** were demonstrated by transfection of mammalian cells containing DNA fragments corresponding to the region of interest. In contrast, the mutant sequence demonstrated loss of binding to nuclear proteins and reduced transcriptional activity. The latter finding was not confined to tissues known to express alpha 1-antitrypsin.(ABSTRACT TRUNCATED AT 250 WORDS)

L4 ANSWER 6 OF 9 MEDLINE on STN DUPLICATE 2
AN 91348975 MEDLINE
DN PubMed ID: 2102901
TI Alpha 1-**antitrypsin** variants produced by recombinant **DNA** : differences in elastase inhibitory activity and resistance to oxidant agents.
AU Luisetti M; Pozzi E; Diomede L; Donnini M; Piccioni P D; Bolzoni G; Peona V; Salmona M
CS Istituto di Tisiologia e Malattie dell' Apparato Respiratorio, IRCCS Policlinico San Matteo, Pavia, Italy.
SO International journal of tissue reactions, (1990) 12 (6) 363-8.
Journal code: 8302116. ISSN: 0250-0868.
CY Switzerland

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199110
ED Entered STN: 19911020
Last Updated on STN: 20000303
Entered Medline: 19911001
AB Inherited or "acquired" deficiency of alpha 1-antitrypsin (believed to be the cause of pulmonary emphysema) will probably be treated in the future by replacement with alpha 1-antitrypsin purified from human plasma or produced by recombinant DNA, which seems promising because it permits site-specific mutagenesis in the oxidizable active site of the normal human alpha 1-antitrypsin. The aim of this in-vitro study was to investigate the elastase inhibitory activity and the resistance to oxidizing agents of normal human alpha 1-antitrypsin, a recombinant yeast-produced variant (VAL 358) and a recombinant E. coli-produced variant (LEU 358). The inhibitors were exposed to chemical oxidants (NCS, H2O2, xanthine/xanthine oxidase, chloramine-T) and to PMA-activated neutrophils. The elastase inhibitory activity was assayed on porcine pancreatic elastase and neutrophil elastase. Normal alpha 1-antitrypsin and VAL 358 variant were good inhibitors of both elastases. LEU 358 variant was the best inhibitor for neutrophil elastase, but it poorly inhibited the porcine pancreatic elastase. Normal alpha 1-antitrypsin was affected by all oxidants; both variants were almost totally resistant to chemical oxidants and to activated neutrophils. We conclude that recombinant alpha 1-antitrypsin variants differ in their elastase inhibitory **activity** and offer **increased** resistance to oxidant agents.

L4 ANSWER 7 OF 9 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AN 91:198889 SCISEARCH
GA The Genuine Article (R) Number: FE691
TI ALPHA1-**ANTITRYPSIN** VARIANTS PRODUCED BY RECOMBINANT-**DNA** - DIFFERENCES IN ELASTASE INHIBITORY ACTIVITY AND RESISTANCE TO OXIDANT AGENTS
AU LUISETTI M (Reprint); POZZI E; DIOMEDE L; DONNINI M; PICCIONI P D; BOLZONI G; PEONA V; SALMONA M
CS POLICLIN SAN MATTEO, IST RIC & CURA CARATTERE SCI, IST TISIOL & MALATTIE APPARATO RESP, I-27100 PAVIA, ITALY (Reprint); UNIV TURIN, CATTEDRA FISIOPATOL RESP, I-10124 TURIN, ITALY; MARIO NEGRI INST PHARMACOL RES, ENZIMOL LAB, I-20157 MILAN, ITALY
CYA ITALY
SO INTERNATIONAL JOURNAL OF TISSUE REACTIONS-EXPERIMENTAL AND CLINICAL ASPECTS, (1990) Vol. 12, No. 6, pp. 363-368.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 27
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Inherited or "acquired" deficiency of alpha 1-antitrypsin (believed to be the cause of pulmonary emphysema) will probably be treated in the future by replacement with alpha 1-antitrypsin purified from human plasma or produced by recombinant DNA, which seems promising because it permits site-specific mutagenesis in the oxidizable active site of the normal human alpha 1-antitrypsin. The aim of this in-vitro study was to investigate the elastase inhibitory activity and the resistance to oxidizing agents of normal human alpha 1-antitrypsin, a recombinant yeast-produced variant (VAL 358) and a recombinant E. coli-produced variant (LEU 358). The inhibitors were exposed to chemical oxidants (NCS, H2O2, xanthinelxanthine oxidase, chloramine-T) and to PMA-activated neutrophils. The elastase inhibitory activity was assayed on porcine pancreatic elastase and neutrophil elastase. Normal alpha 1-antitrypsin and VAL 358 variant were good inhibitors of both elastases. LEU 358 variant was the best inhibitor for neutrophil elastase, but it poorly

inhibited the porcine pancreatic elastase. Normal alpha 1-antitrypsin was affected by all oxidants; both variants were almost totally resistant to chemical oxidants and to activated neutrophils. We conclude that recombinant alpha 1-antitrypsin variants differ in their elastase inhibitory **activity** and offer **increased** resistance to oxidant agents.

L4 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 3
AN 88216579 MEDLINE
DN PubMed ID: 2835657
TI A cell-specific enhancer of the mouse alpha 1-antitrypsin gene has multiple functional regions and corresponding protein-binding sites.
AU Grayson D R; Costa R H; Xanthopoulos K G; Darnell J E Jr
CS Rockefeller University, New York, New York 10021.
NC CA 160006-14 (NCI)
CA 18213-11 (NCI)
GM 1066-02 (NIGMS)
+
SO Molecular and cellular biology, (1988 Mar) 8 (3) 1055-66.
Journal code: 8109087. ISSN: 0270-7306.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198806
ED Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880614
AB We have previously described the isolation and characterization of genomic clones corresponding to the mouse alpha 1-**antitrypsin** gene (Krauter et al., **DNA** 5:29-36, 1986). In this report, we have analyzed the DNA sequences upstream of the RNA start site that direct hepatoma cell-specific expression of this gene when incorporated into recombinant plasmids. The 160 nucleotides 5' to the cap site direct low-level expression in hepatoma cells, and sequences between -520 and -160 bp upstream of the RNA start site functioned as a cell-specific enhancer of expression both with the alpha 1-antitrypsin promoter and when combined with a functional beta-globin promoter. Within the enhancer region, three binding sites for proteins present in hepatoma nuclear extracts were identified. The location of each site was positioned, using both methylation protection and methylation interference experiments. Each protein-binding site correlated with a functionally important region necessary for full **enhancer activity**. These experiments demonstrated a complex arrangement of regulatory elements comprising the alpha 1-antitrypsin enhancer. Significant qualitative differences exist between the findings presented here and the cis-acting elements operative in regulating expression of the human alpha 1-antitrypsin gene (Ciliberto et al., Cell 41:531-540, 1985; De Simone et al., EMBO J. 6:2759-2766, 1987).

L4 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1986:63387 CAPLUS
DN 104:63387
TI α-1- **Antitrypsin** mutants, **DNA** coding for them and therapeutic formulations using these mutants
IN Insley, Margaret Y.; Kawasaki, Glenn Hitoshi
PA Zymogenetics, Inc., USA
SO Eur. Pat. Appl., 31 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI	EP 155188	A2	19850918	EP 1985-301790	19850314
	EP 155188	A3	19860813		
	EP 155188	B1	19931229		
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	US 4711848	A	19871208	US 1985-709382	19850307
	AU 8539819	A1	19850919	AU 1985-39819	19850313
	AU 593766	B2	19900222		
	JP 61012289	A2	19860120	JP 1985-51553	19850314
	JP 2539781	B2	19961002		
	EP 566158	A1	19931020	EP 1993-107971	19850314
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	AT 99358	E	19940115	AT 1985-301790	19850314
	JP 10113193	A2	19980506	JP 1997-275648	19850314
	JP 06105689	A2	19940419	JP 1993-115129	19930406
	JP 2750257	B2	19980513		
PRAI	US 1984-589410		19840314		
	US 1985-709382		19850307		
	EP 1985-301790		19850314		
	JP 1993-115129		19850314		

AB The gene for human α1-antitrypsin (I) [9041-92-3] is subjected to site-directed mutagenesis and cloning to produce a protein with **enhanced** stability or antithrombin [9000-94-6] **activity** . Substitution of methionine-358 in the active site with other amino acids protects the protein from oxidation Substitution of lysine for glutamic acid-342 produces the Z-allele variant, which is nonimmunogenic when administered to patients homozygous for the Z-allele. [Arg358]I has antithrombin activity and maybe useful as an anticoagulant. For example, site-directed mutagenesis was carried out by annealing an oligonucleotide containing a desired mutant codon for either position 342 or 358, together with the universal primer of phage M13, to single-stranded recombinant M13 DNA containing the wild-type I gene. Active phage was produced by oligonucleotide extension, and ligation, and transfection into competent Escherichia coli K12. The mutant I coding region was removed by digestion with BamHI and PstI and inserted into an expression vector, e.g. M13 mp 10.

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